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Involvement of *ATM* Missense Variants and Mutations in a Series of Unselected Breast Cancer Cases

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It has been proposed that women carrying heterozygous mutations of the *ATM* gene could be at increased risk of developing breast cancer. However, data in the literature are contrasting and no firm conclusion has been reached. Our aim was to verify whether *ATM* inactivation could play a role in breast tumor development. Following the classical tumor suppressor inactivation scheme, tumors showing loss of heterozygosity (LOH) at the *ATM* locus should present an increased proportion of mutated *ATM* forms. We screened a cohort of 173 nonselected primary breast tumors for LOH in a 4 cM region at 11q23 spanning the *ATM* gene. We analyzed 25 tumors presenting LOH within the *ATM* locus for mutations in the *ATM* coding sequence using an RT-PCR-SSCP approach. Five patients were found to bear a coding missense variant, out of which four corresponded to a frequent polymorphism in exon 39. One patient presented a previously unreported variant in exon 19 (2614C>T) resulting in a nonconservative change (Pro>Ser) at aa 872. This variant was not found in any of the other 172 patients nor in 63 healthy controls tested, indicating that it is a rare *ATM* variant. LOH involved the *ATM* wild-type allele in the tumor presenting variant 2614. However, because the *ATM* gene presents a relatively large number of rare coding polymorphism it is difficult, in the absence of familial data, to be conclusive on the significance of this variant. Searching for further variants in exons 19 and 39 in the whole set of 173 breast tumors, we found one tumor showing an acquired deletion of four bases in the *ATM* gene. Somatic mutations affecting the *ATM* gene thus seem rare in breast cancer. In our cohort of breast cancer patients, tumors presenting LOH at the *ATM* locus did not show an increased frequency of sequence variants. Furthermore, allelic imbalance profiles in a 4-cM region of chromosome arm 11q spanning the *ATM* locus revealed that hot spots of LOH were more likely to correspond to a region localized telomeric to the gene. Therefore, these data suggest that other target genes for genetic inactivation exist in the 11q23 region.

INTRODUCTION

The *ATM* gene is the genetic determinant of ataxia telangiectasia, a rare recessive disorder characterized by immunological deficiencies, hypersensitivity to ionizing radiation, and predisposition to cancer. *ATM* codes for a large nuclear phosphoprotein which plays an important role in double-stranded DNA break signaling and cell cycle checkpoints. The ATM protein has been shown to interact with proteins or protein complexes like BRCA1, ABL, TP53, and Nibrin/MRE11/RAD50 (for review, see Canman and Lim, 1998; Rotman and Shiloh, 1998). Mutations in the *ATM* gene have been linked to chromosomal instability, to defects in cell cycle checkpoints, and programmed cell death. While phenotypic manifestations in *ATM* homozygotes or compound heterozygotes are known to be severe, heterozygous carriers are mostly asymptomatic. However, a number of studies have suggested that obligate heterozygous carriers of *ATM* mutations could be at increased risk of developing cancer, and more specifically breast cancer in women. The risk of developing breast cancer has been estimated to be 3 to 5 times higher than in the general population. Knowing that the incidence of A-T heterozygotes in the general population is estimated to be in the range 0.5–1%, these data bear some significance in terms of public health. Indeed, the proportion of *ATM* heterozygotes has been estimated to rise to 4% in breast cancer patients (Easton, 1994).

The *ATM* gene maps to chromosome band 11q23, which is a region frequently involved in chromosomal losses in breast cancer (Carter et al., 1994; Hampton et al., 1994; Negrini et al., 1995; Laake et al., 1999). Furthermore, the *ATM* gene has been shown to be inactivated, either by somatic

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mutations or by chromosomal rearrangements, in a number of B- or T-cell leukemia cases (Stilgenbauer et al., 1997; Vorechovsky et al., 1997; Stoppa-Lyonnet et al., 1998; Yuille et al., 1998; Stankovic et al., 1999). These observations supported the idea that the *ATM* gene may act as a tumor suppressor and its inactivation may be part of the tumorigenic process. According to the classic tumor suppressor inactivation scheme, two events are required: loss of the wild-type allele and retention of the mutated allele (Knudson, 1971). Given that the mutation can either be acquired or inherited, we screened a panel of 173 breast tumors for LOH in the 11q23 region. The selected CA repeat markers span the *ATM* locus as well as several Mb telomeric to this gene. Out of 41 tumors presenting LOH in the *ATM* locus, we analyzed 25 for mutations in the *ATM* coding sequence by the single-stranded conformation polymorphism (SSCP) method. Furthermore, we searched for exon-skipping mutants using a series of overlapping PCR primers. To check whether the variants detected corresponded to mutations or rare sequence polymorphisms, we extended the analysis to the entire cohort of 173 patients and compared the incidence to that observed in a set of 63 sex- and age-matched healthy blood donors.

MATERIALS AND METHODS

Biological Samples

One hundred seventy-three primary breast tumors and matching blood samples were collected at the Regional Cancer Center in Montpellier, France (C.R.L.C. Val d'Aurelle). All tumors were primaries with no presurgical treatment. The distribution according to clinicopathological parameters was as follows: invasive ductal, 131; invasive lobular, 32; other types, 11; Scarf, Bloom, and Richardson Grading I, 17; Grade II, 73; Grade III, 65; negative lymph nodes, 96; positive, 68; estrogen receptor-positive, 110; negative, 63; progesterone receptor positive, 115; negative, 57; under 50 years, 54; over 50, 119. Tissues were snap-frozen in liquid nitrogen upon surgical removal and subsequently kept at −80°C until DNA or RNA extraction. White blood cell nuclei were isolated from blood by hypotonic lysis of red cells and stored at −20°C until DNA extraction.

The healthy control population was composed of 63 female bone marrow donors. This set of individuals was selected on the basis of their age distribution.

Nucleic Acid Extraction

The isolation of genomic DNA was performed as described previously (Rodriguez et al., 2000). Extraction of RNA was performed with guanidinium thiocyanate followed by centrifugation on a cesium chloride gradient.

cDNA Synthesis

cDNA synthesis was performed using 2 μg of total cellular RNA and 500 ng of oligo(dT) primer, using Expand Reverse transcriptase (Roche, Meylan France) according to the manufacturer's recommendations.

Primers

CA repeat microsatellite sequences were obtained from data stored in the Genome Database (http://www.gdb.org). All the CA repeat markers included in this study mapped to the 11q23 region. Reverse primers used to amplify microsatellite sequences were fluorescently labeled with HEX, FAM, or TET dye. Primers designed on *ATM* cDNA are listed in Table 1; they were used in different combinations, depending on the fragment size required.

PCR Amplification

Two μl of reverse transcription products or 25 ng of genomic DNA were used as template for a 35-cycle PCR amplification using AmpliTaq (Applied Biosystems, Courtaboeuf, France) under standard conditions.

Allelotyping Analysis

PCR products were mixed in loading buffer containing fluorescent DNA molecular weight marker (GS350 TAMRA, Applied Biosystems), run on 4% polyacrylamide denaturing gel for 2 hr at 3000 V using a 377 DNA sequencer, and automatically analyzed by Genescan analysis software. A decrease of one of the allelical peak of at least 20% was considered an allelic imbalance. Samples presenting AI were routinely analyzed twice, and only those which could be reproduced in all experiments were included in the study.

SSCP Analysis and Sequencing

Eight of the PCR products were digested by a restriction enzyme prior to SSCP analysis in order to obtain fragments ranging between 305 and 374 bp (cf. Table 1). SSCP analysis was performed either on nondenaturing MDE gel (Tebu, Le Perray en Yvelines, France) containing 5% glycerol as

previously described, or on excelgel or cleangel (Amersham-Pharmacia Biotech, Saclay, France) and run on multiphor apparatus according to the manufacturer's recommendations. The band shift fragments were eluted from the gel as previously described, reamplified, and sequenced using dRhodamine terminator cycle sequencing kit (Applied Biosystems) according to the manufacturer's recommendations.

RESULTS

LOH in the 11q23 Region in Breast Tumors

We analyzed a series of 173 pairs of breast tumor and cognate normal lymphocyte DNAs for LOH in the 11q23 region, where the *ATM* gene maps. Eight CA repeat markers selected from the Genome Database were included in this study (Table 1). These markers span approximately 4 cM, which, according to GDB, should correspond to 6 Mb. Markers *D11S1343* and *D11S1778* flank the *ATM* locus on either side, while *D11S2179* is intragenic, being localized in intron 61 (Platzer et al., 1997). Out of the 173 tumors screened, 103 presented allelic imbalance for at least one of the tested markers. The frequency of imbalance varied from one locus to another, showing a minimum of 34% at *D11S1343* and a maximum of 53.8% at *D11S1284* (Table 1). Three peaks were observed at *D11S1778*, *D11S2000*, and *D11S1284* which showed, respectively, imbalance in 45.5%, 47.8%, and 53.8% of the cases. Fifty-three tumors showed imbalance at all informative markers. Other tumors showed complex profiles of allelic losses, including zebra patterns or interstitial losses (Fig. 1A). A close examination of the tumors with interstitial losses suggested the existence of at least three minimal regions of overlap: *D11S1778*, *D11S2000*, and *D11S2003*. Interestingly, eight tumors showed conservation of heterozygosity at *D11S2179* and hemizygosity at *D11S1778*, suggesting that the *ATM* gene could be excluded from the region of loss and that the breakpoint was near its 3′ end (Fig. 1A).

We were interested in tumors showing LOH within the *ATM* gene and therefore selected tumors showing allelic imbalance at *D11S2179*. Out of 99 informative cases, 41 presented imbalance at this marker. In a number of these tumors the imbalance extended to other markers tested (Fig. 1B). Out of 41 specimen showing LOH at *D11S2179*, tumor tissue in sufficient quantities for RNA extraction was available in 25 cases.

TABLE 1. Frequency of Allelic Imbalance at Markers Spanning the 11q23 Region

Locus	Number of informative tumors	Allelic imbalance (% of informative tumors showing imbalance)
D11S1343	103	34.0
D11S2179	99	41.4
D11S1778	123	45.5←
D11S1294	126	40.5
D11S2000	136	47.8←
D11S1818	103	39.8
D11S2003	121	45.5
D11S1284	65	53.8←

Modifications in the *ATM* Gene

We searched for mutations in the *ATM* coding sequence using an RT-PCR approach. RNAs extracted from the 25 selected tumors were reverse-transcribed and cDNA used as a template for subsequent PCR amplifications. Two types of events were searched for: 1) aberrant transcripts resulting from exon skipping, and 2) discrete sequence variants such as nucleotide substitutions, small insertions, or deletions.

In order to detect aberrant transcripts possibly resulting from splicing errors, we subdivided the *ATM* transcript into eight overlapping PCR fragments of approximately 2,200 bp (Table 2). Each PCR fragment spanned several exons. Exon skipping should have resulted in shortened PCR amplimers easily detected on a 1% agarose gel. Our data did not support the existence of such exon skipping mutants in the *ATM* transcripts analyzed, since none of the samples presented an abnormally sized band in any of the eight overlapping PCR fragments.

We used PCR-SSCP to detect discrete mutations in the coding sequence of *ATM*. To this end, 24 overlapping PCR fragments were designed in order to amplify products with an average size of 350 bp (Table 2). Five tumors showed aberrant band shifts. Sequencing of variant bands revealed nucleotide substitutions leading to nonconservative amino acid changes in five tumors. Mutations corresponded to a CCT→TCT transition at position 2614 (exon 19) leading to the change of Pro into Ser at amino acid residue 872 in one tumor, and a GAT→AAT substitution at position 5557 (exon 38), leading to the replacement of Asp into Asn at aa 1853 in four tumors (Fig. 1B). To assess the acquired or inherited nature of these modifications we performed PCR-SSCP analysis on DNA from both the tumor and matched normal lymphocytes (Fig. 2). All variants were detected in blood samples showing their germline origin.

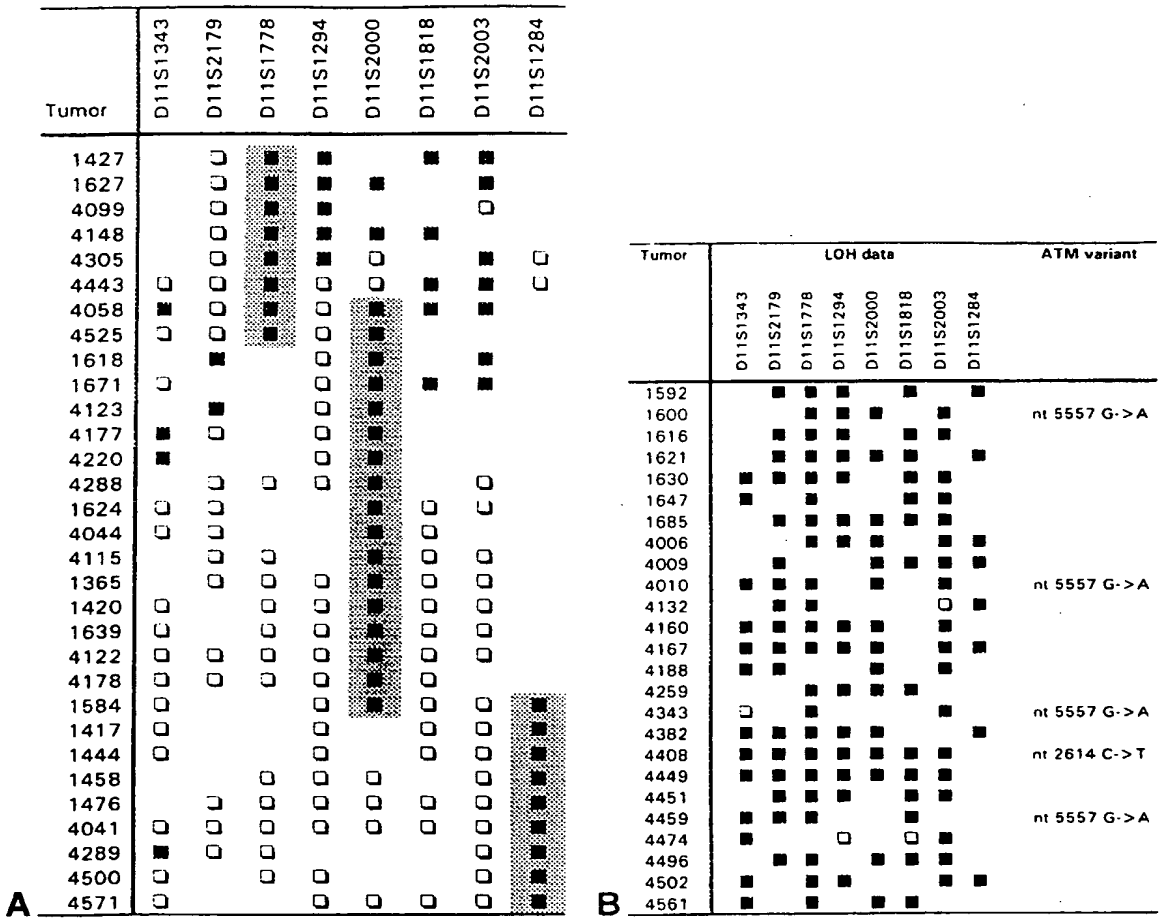

Figure 1. LOH in a 4-cM region at 11q23 spanning the ATM gene. **A:** Representative patterns of LOH in a subset of tumors. **B:** LOH patterns of the tumors analyzed by PCR-SSCP, substitutions detected in the *ATM* coding sequence are shown on the right. Rows correspond to individual tumors, sample numbers are indicated on the left. Results were coded as follows: (open square) retention of both alleles, (solid square) AI. No symbol corresponds to an absence of information due to the homozygosity of the markers. Minimal regions were shaded. Markers are ordered from centromere to telomere.

Polymorphisms, Rare Variants, or Mutations

Because a number of variants observed in the *ATM* gene have been found to correspond to sequence polymorphisms (Castellvi-Bel et al., 1999) rather than disease-associated mutations, we extended our search for variants in exons 19 and 39 and analyzed 173 breast tumors previously tested for LOH. PCR-SSCP analysis was performed at the DNA level using adapted PCR primer pairs. As shown in Figure 3, variant C>T 2614 was not found in any other of the 172 breast tumors. Three additional variants were found in exon 19, out of which two (tumors 4177 and 4178) corresponded to a T>C substitution at position 2572, resulting in a Phe>Leu change at aa 858. The third one, observed in tumor 1427, corresponded to a 4 bp deletion (2563 delATGA) resulting in a stop codon at position 878. Variant 2572T>C was present in both normal and tumor DNA, indicating its germline origin. Remarkably, variant 2563 delATGA was found only in tumor DNA, indicating that it had been acquired during the tumorigenic process. SSCP analysis of exon 39 in the cohort of 173 breast tumor DNAs revealed the existence 31 additional patients bearing the 5557 G>A variant, among which three were homozygous. This brought the number of patients presenting this polymorphism to 35/173 and its frequency to 11.0% (38/346 chromosomes) in our breast cancer cohort.

Variants in exon 19 and 39 were then searched in the control population of 63 age-matched healthy women of identical geographical origin. As shown

TABLE 2. Oligonucleotide Primers Used for ATM Amplifications

	Forward primers	Reverse primers	Amplimer position	Restriction enzyme
1	GCACAGTGATGTGTGTTCT	GCTTGTGTTGAGGCTGAT	158–452	
2	GAATAGCAAAACCAAATGTAT	GCATGAATTATTCTAGCCAC	416–767	
3	CCTTCACAAGATGTTCATACAG	AGCACCTTTTTCTTGGGT	721–1095	
4	CCATCATCCGAAAGGAGCC	ATTACTTCCCAGCCTAGTTC	1056–1376	
5	GTGTCCGTTGCAAAAGGAAC	CACCAAATTTTATTCCAGAG	1331–1655	
6	GAGGTCAAACCTAGAAAGC	GTGCTATTTTCTAAGTCACCC	1591–967	
7	GGCTCTTATTCTATCAGTTAG	CAGAAGACAGCCATCCAGT	1925–2274	
8	CTCTCCACCAGAATCTCAAG	CAACGTGTACATAGCTGCA	2231–2546	
9	GAGGAATTCAGAATTGGTTCC	CACCTATGGTACTTTGGCTC	2494–2830	
10	GTGTTAGTGATCCAAACGAAC	CAAGAACATCTTCCATTGGC	2783–3091	
11	GGAGCTTCCTGGAGAACAG	CCATTACATTAAGAATGGCC	3045–3382	
12	CTTTGCTTGAGGCTGATCC	GCCTGTTTTTCGCAGATAG	3332–3704	
13	ACTGACGTTGATAGCTGTGG	CCACTTCACCTCATCAAAATG	3651–3999	
14	GATCTTGTTATAAGGTTTTGATTCC	GCACTACAATTTGCTGGCTC	3935–4265	
15	GACGTTACATGAGCCAGC	ACATGGTCAGGAAAAGGATCT	4236–4895	*Taq* I
16	TAACAAGGATAATGAAAAGCTC	CTTTCTCAAATCTGGGTACTTC	4833–5536	*Xba* I
17	ACAACAGATCCAATGCTGGC	AGTTGTACTCTGGCTTCCTTC	5455–6144	*Pvu* II
18	TACAGCTTTACTCTATGCAG	CAATGCTTTCCAGCTCTCCA	6045–6745	*Stu* I
19	GCCAGAGTAAAAGAAGTGGAA	CCATTTCTTAGCTCATCACTAC	6637–7334	*Ssp* I
20	GGTTAGCAGAAACGTGCTTAG	CATCTCTGTTTGCATTTGCTAAG	7220–7898	*ApaL* I
21	CCTAGGATTTCATGAAGTCCTC	CATTCAAGAACACCACTTCGC	7791–8495	*Ssp* I
22	CTGCAGAGAAACACGGAAAC	TAGAAGGACCTCTACAATGG	8404–9048	*Bam* HI
23	GTCTTCAGAAGATGCTGTGA	CATTAACACACGTTCAGCTAC	8965–9222	
24	GATATTGACCAGAGTTTCGAC	CTAAAGGCTGAATGAAAGGG	9178–9398	
25	CATCTTTGTTTCTCTTCCTTGAAG	CCCAAGTAGTAAATATGTATTTAC	EX 19	
26	CATTTTCTAATCCCTTCTTTCTAG	ATATCAGACTAATTAATCTTCTTAC	EX 39	

Primer pairs used to amplified fragments for SSCP analysis are noted on each row and regions amplified are specified in column 4. Some of the PCR products were digested by the restriction enzymes indicated in the right column prior to SSCP analysis. The two last rows correspond to primers used on genomic DNA. Exon skipping analysis was done using following primer pairs: 1F-8R, 5F-11R, 8F-14R, 12F-16R, 15F-19R, 17F-20R, 19F-21R, 21F-24R.

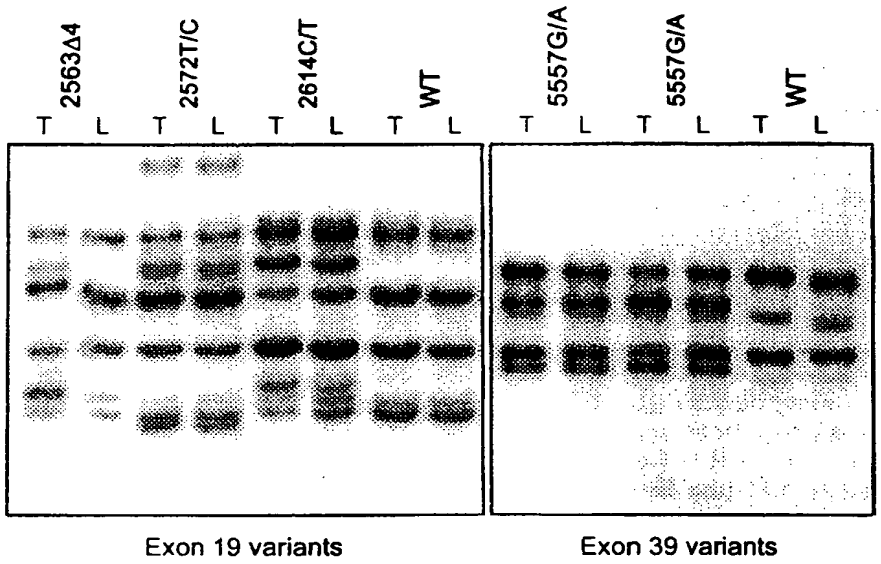

Figure 2. PCR SSCP analysis of the ATM gene. PCR fragments spanning exon 19 and exon 39 were heat denatured and run on 6% polyacrylamide gels containing 5% glycerol. Asterisks indicate aberrantly migrating bands. T: tumor, L: lymphocyte, WT: wild type.

in Table 3, none of them presented a variant in exon 19, whereas variant 5557 G>A was found in 14/63 normal controls among which three were homozygous. Its frequency among the controls was 13.5% (17/126 chromosomes), similar to that found in the cancer cohort.

tumor ID	D11S1343 Allele a	D11S1343 Allele b	D11S2179 Allele a	D11S2179 Allele b	D11S1778 Allele a	D11S1778 Allele b	LOH D11S1343	D11S2179	D11S1778	D11S1294	D11S2000	D11S1818	D11S2003	D11S1284	ATM Variants exon 19	ATM Variants exon 39	Loss of ATM allele on SSCP
1427	227		139	141	138	152 (-)									2563 delATGA	-	NA
4177	227	254 (-)	135	137	138										2572 T>C	-	no loss
4178	227	239	137	141	136	142									2572 T>C	5557 G>A	no loss
4408	227 (-)	235	137	141 (-)	138	147 (-)									2614 C>T	-	wt lost
1408	227	239	141		142	154									-	5557 G>A	no loss
1600	227		137		138	158 (-)									-	5557 G>A	wt lost
1721	227	233	139	143	138	142									-	5557 G>A	no loss
4010	227	233 (-)	139	141 (-)	140	155 (-)									-	5557 G>A	wt lost
4022	227		135		138	140									-	5557 G>A	wt lost
4044	227	233	137	145	138										-	5557 G>A	no loss
4115	227		137	139	142	157									-	5557 G>A	no loss
4124	227	239	139		140	153									-	5557 G>A	no loss
4150	228	240	135	139	138	144									-	5557 G>A	no loss
4168	233	241 (-)	137 (-)	143	138 (-)	144									-	5557 G>A	wt lost
4169	227	249	139	143	138	142									-	5557 G>A	no loss
4199	239		140	142	142	157									-	5557 G>A	no loss
4201	227		139	143	138	142									-	5557 G>A	no loss
4208	227		133 (-)	137	138	142 (-)									-	5557 G>A	wt lost
4209	227		141	145 (-)	142 (-)	148									-	5557 G>A	variant lost
4212	233	239	135	143	142										-	5557 G>A/5557 G>A	NA
4221	227		137	141	142	151									-	5557 G>A	no loss
4230	227		141		144	153									-	5557 G>A	no loss
4246	227	233	137	143	138	142									-	5557 G>A	no loss
4258	227		137 (-)	141	142 (-)	155									-	5557 G>A	variant lost
4261	227	239	137	141	138	157									-	5557 G>A	no loss
4313	233		139	145 (-)	138	142 (-)									-	5557 G>A	variant lost
4343	227	233	137		138	142 (-)									-	5557 G>A	variant lost
4349	227	255	139	143	138	143									-	5557 G>A	no loss
4380	225	232	141	145	142										-	5557 G>A/5557 G>A	NA
4383	230	240	142		142	151									-	5557 G>A	no loss
4385	223	239	137	145	138	142									-	5557 G>A	no loss
4388	226	234 (-)	139		142	155 (-)									-	5557 G>A	wt lost
4441	233 (-)	239	141		140	153 (-)									-	5557 G>A	wt lost
4442	227 (-)	229	133 (-)	143	138 (-)	142									-	5557 G>A	wt lost
4459	227 (-)		135 (-)	137	138 (-)	142									-	5557 G>A	wt lost
4483	234	244	139		138	140									-	5557 G>A	no loss
4525	227	239	139	143	138	142									-	5557 G>A	no loss
4680	230		143		143										-	5557 G>A/5557 G>A	NA

Figure 3. Allele distribution at markers flanking the ATM gene and LOH in tumors presenting polymorphisms or sequence variants.

Loss of the Wild-Type Allele in Tumors Showing *ATM* Variants

In order to find out whether the occurrence of an *ATM* variant in breast tumors coincided with LOH at this locus and, if so, which of the *ATM* alleles had been lost, the wild type or the variant. LOH status of markers *D11S1343*, *D11S2179*, and *D11S1778* was investigated in these tumors. To determine which *ATM* allele had been lost, tumor and normal DNAs from the same patient were analyzed in parallel by SSCP. The relative intensities of both wild type and variant alleles were determined on the SSCP gels. As shown in Figure 2, no consistent pattern of allele loss was observed. Out of the four patients showing variants in exon 19, only case 4408 (2614 C>T) presented LOH at *D11S2179* and loss of the *ATM* wild-type allele in the tumor. In contrast, tumor 1427, showing an acquired mutation (2563 del ATGA), did not present a reduction of the *ATM* wild-type allele and was heterozygous at *D11S2179*. LOH profiles in tumors with *ATM* variant 5557 were not consistent. *D11S2179* showed LOH in 9/24 informative tumors. SSCP analysis confirmed the frequent retention of both alleles. In tumors showing ploidy reduction, the lost allele could alternatively correspond to the wild type or the variant (Fig. 3).

DISCUSSION

The *ATM* gene displays a number of features that make it an ideal culprit in the search for cancer genes. The first indication comes from the very

in any additional patient. Nor did we detect it in a control series of 63 age- and sex-matched healthy blood donors. Three other tumors presented sequence variants in exon 19 of the *ATM* gene. Two corresponded to a 2572T>C variant previously reported in two studies on breast cancer patients (Vorechovsky et al., 1996b; Izatt et al., 1999). It is therefore probable that this variant corresponds to a rare *ATM* coding polymorphism whose involvement in breast carcinogenesis seems doubtful. Indeed, neither of the two tumors presenting this variant in our study showed LOH at the *ATM* locus. Furthermore, this variant did not cosegregate with breast cancer (Izatt et al., 1999). The relatively frequent occurrence of coding polymorphisms in the *ATM* gene makes it difficult to determine whether missense variants are associated with breast cancer development. Therefore, the significance of variant 2614C>T is uncertain. This variant was not reported in the ATM database and the tumor presenting this variant lost the wild-type allele. However, familial segregation data will be needed.

Characteristics of the third variant detected were remarkable, since it corresponded to an acquired deletion of four bases at base 2563 (del ATGA). This is the first time, to our knowledge, that an acquired mutation in the *ATM* gene is reported in breast cancer. This finding could, thus, be in favor of the *ATM* gene being inactivated during the course of tumor development in breast cancer. Although the tumor showing this mutation had retained both *ATM* alleles, phenotypical consequences, due to haploinsufficiency or a dominant negative effect, cannot be excluded. As a matter of fact, a number of TP53 mutations have been observed in tumors presenting retention of the wild-type allele (Mazars et al., 1992).

In the present study, tumors selected for LOH within the *ATM* gene did not show a clear increase in sequence variants, with only 1/25 cases displaying a potentially significant sequence variant. The frequency of *ATM* heterozygotes is estimated to be 0.5–1% in the general population and, possibly, up to 4% among breast cancer patients. Our study design was based on a preselection of tumors presenting LOH, in which tumors bearing *ATM* variants should have been concentrated. Thus, starting from a population of 173 tumors, we should have found 3–7 *ATM* mutants. Instead, we found only two mutants variants, if we include the 2614C>T and 2563 (del ATGA) variants. These results could be interpreted in two ways: 1) *ATM* variants do not play the expected role in breast tumorigenesis, or 2) *ATM* variants could be of low penetrance and may not consistently follow the classical two hit tumor suppressor inactivation scheme. Recent data by Lu et al. (2001) showing that the *ATM* gene is disrupted by large deletions in two breast cancer cell lines and one primary tumor suggest that the *ATM* gene can also be inactivated by gross rearrangement of sporadic origin. Furthermore, allelic imbalance profiles in a 4-cM region spanning the *ATM* locus of chromosome arm 11q revealed that hot spots of LOH were more likely to correspond to a region localized telomeric to *ATM*. These data support reported results (Kerangueven et al., 1997) suggesting that the *ATM* gene is not the main target of genetic inactivation in the region and that other candidate tumor suppressors may have to be considered.

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